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FINAL PROGRESS REPORT

GRANT # DAAG55-97-1-0134

**TITLE: Sensitive, Selective Fluorescent Probes for the
Detection and Identification of Nucleic Acids**

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BODY - DESCRIPTION OF SCIENTIFIC RESULTS

GRANT TITLE: Sensitive, Selective Fluorescent Probes for the Detection and Identification of Nucleic Acids

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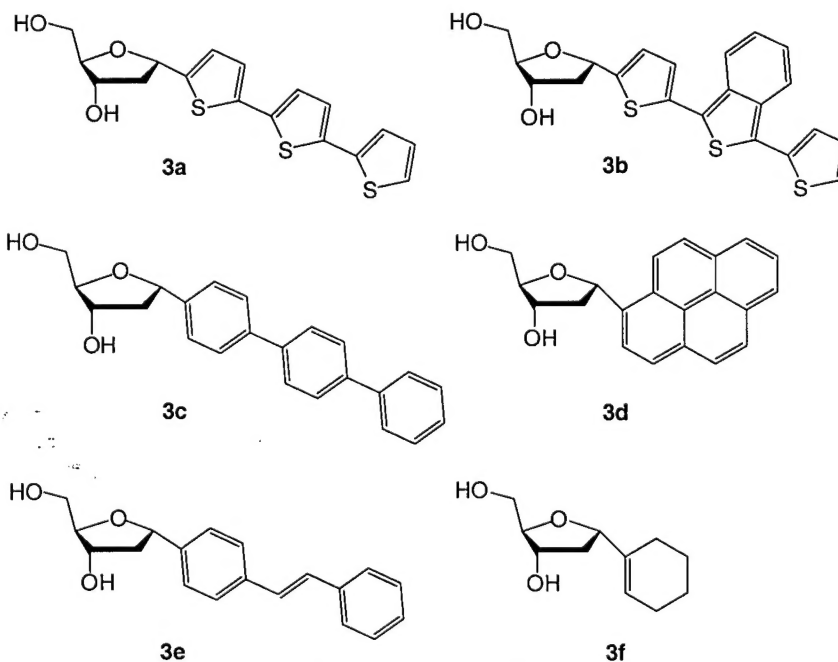
STATEMENT OF THE PROBLEM STUDIED

The long-term goals of this project are to develop new strategies for the use of oligonucleotides tagged with multiple fluorescent labels as hybridization probes of specific nucleic acid sequences. First, we have designed (and continue to design) new classes of fluorescent labels for DNA. Second, we aim to develop novel modes of interaction between fluorescent labels, leading to color changes on detection of nucleic acids. Finally, we aim to develop new methods for increasing specificity of nucleic acid recognition.

SUMMARY OF RESULTS

This grant allowed us to develop several new strategies for the detection of RNAs and DNAs by use of fluorescence color changes. In the grant period we achieved notable new developments both in fluorescent labels as well as in new methods for increasing specificity of recognition.

New fluorophores for DNA labeling. We have been focusing strongly on methods for



incorporation of multiple fluorophores into DNA backbones. We use multiple fluorophores to increase molar absorptivity of the DNA label, so that the DNA can be detected at low concentrations. We have focused on simple aromatic hydrocarbons and related fluorophores. In 1999 we completed the synthesis of several new fluorescent nucleosides (see above) for future incorporation into DNA strands. We plan to investigate their fluorescence properties when multiple dyes are placed in a single DNA. We hope to identify cases which give brighter fluorescence with multiple substitution, and/or which give altered emission wavelength with multiple substitution.

"Autoligation" for increasing specificity. Most DNA detection methods rely on the specificity of DNA base pairing to give a signal upon hybridization. For example, fluorescent-labeled DNA probes are very commonly used to detect complementary RNAs and DNAs. However, there are problems with this simple approach that lead to relatively low specificity in sequence detection. In general, probes are commonly ca. 20 nucleotides long, so that single sites in large genomes are specified. Unfortunately, single base mismatches with a 20mer are difficult to distinguish, because the probe is not disrupted much by a single perturbation out of 20 base pairs.

We therefore developed a new approach to detection by hybridization which dramatically increases specificity. This approach involves an autoligation reaction between two shorter chemically modified DNAs. It is termed autoligation because the probes ligate themselves together without need for enzyme or added reagents. We commonly use two probes of length 7 and 13 nucleotides. Because they are short, their binding is highly specific and easily senses single base changes in the target (paper 3 above). We design the probes to bind directly adjacent to one another on the target DNA or RNA. When perfectly complementary at adjacent sites, the probes ligate themselves together to make a 20mer product strand. We showed that when only one base is changed in the target, the rate of ligation slows 180-fold. This level of selectivity is higher than commonly used ligase enzymes. Thus we consider this autoligation approach to be very promising for the detection of genetic sequences in solution or in microarrays. Future work will address methods for reporting on the ligation reaction, so that the product can be easily detected.

A new strategy for faster ligation of DNA. We developed improved chemistry that allows for more rapid ligations of DNA strands. This involves the switching of selenium for sulfur. This new reaction is four-fold faster than the previous sulfur ligation.

Application of a new pyrene nucleoside fluorophore in the detection of DNA damage. We previously described a novel nucleoside in which the fluorophore pyrene replaces the DNA base. We synthesized the nucleoside triphosphate derivative of this nucleoside and asked whether it could act as a substrate of DNA polymerase enzymes. Ordinarily this would not be thought possible, since this compound lacks Watson-Crick hydrogen bonding ability and does not resemble standard purines or

pyrimidines. However, our lab has shown that these features are actually not requirements for polymerase enzymes, and that what is most important is whether a pair of bases can actually fit within the helix (the "shape matching" hypothesis).

Models suggested that pyrene would fit well at sites where a DNA base is missing. Such "abasic" sites are the most common form of DNA damage in a cell. We therefore tested whether polymerases could insert this fluorescent nucleotide opposite abasic sites. We found that not only did it work, but it did so with high efficiency and specificity (paper 1 above). It is possible that this or related compounds could be a useful tool in biomedical research, for identifying sites and origins of DNA damage.

PUBLICATIONS resulting from grant (already reported in Interim reports):

1. Tracy J. Matray and Eric T. Kool, A specific partner for abasic damage in DNA, *Nature* **1999**, 399, 704-708.
2. Christoph Strässler, Newton E. Davis, Eric T. Kool, Novel nucleoside analogues with fluorophores replacing the DNA base, *Helvetica Chimica Acta* **1999**, 82, 1260.
3. Yanzheng Xu and Eric T. Kool, High sequence fidelity in nonenzymatic autoligation of oligodeoxynucleotides, *Nucleic Acids Res.* **1999**, 27, 875-881.
4. Yanzheng Xu and Eric T. Kool, Rapid, selective selenium-mediated autoligation of DNA strands, *J. Am. Chem. Soc.* **2000**, 122, 9040-9041.

FUTURE PLANS

A new ARO grant (DAMD17-98-1-8239-P00001) supports our ongoing work at Stanford. The project is moving forward now. We are working to develop new fluorescent nucleosides that can interact with one another to give color changes. Our chief goals here are (1) development of longer wavelength emitters (yellow and red), and (2) to make a library of several useful fluorophores for general application in sequence detection. Second, we plan to further develop the autoligation reaction for application to nucleic acid detection in human tissue samples.

SCIENTIFIC PERSONNEL AND DEGREES

Graduate students supported: Newton E. Davis. Postdoc: Dr. Tracy Matray. No degrees awarded.

INVENTIONS AND TECHNOLOGY TRANSFER

(this was already reported to ARO in an Interim report)

Several new fluorescent nucleosides were developed (see reference 2 above). They were incorporated into a previously described invention (the pyrene nucleoside). These have all been licensed to a biotechnology company (Intergen), where they are being developed as reporters of nucleic acid detection.